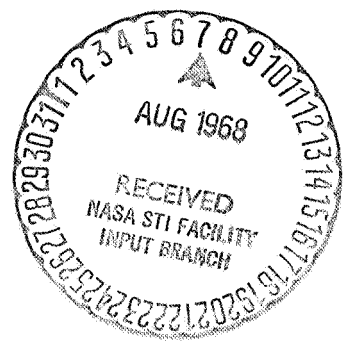


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JET PROPULSION LABORATORY
 CALIFORNIA INSTITUTE OF TECHNOLOGY
 PASADENA, CALIFORNIA

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PROGRESS REPORT
MICROORGANISM STUDY, CIT CONTRACT NO. 950783
Systematic Description and Key to Isolants
From Mexico
Professor W. B. Bollen, Microbiologist
Oregon State University
Corvallis, Oregon
June 1, 1968

This work was performed for the Jet Propulsion Laboratory,
California Institute of Technology, sponsored by the
National Aeronautics and Space Administration under
Contract NAS7-100.

Preface

This report identifies isolants from soils of Mexico. Of the seven isolants one, 250 Aa, is an actinomycete; the remaining six are bacteria and are presented in the following report. A dichotomous key and a list of the isolants and species designations precede the Descriptive Charts for each isolant. A photomicrograph for the one non-viable isolant, 250 Ba, is also included.

The bacterial isolants are all species of the genera Bacillus. Isolant numbers 250 Bb, 251 Aa and 251 Ab are Bacillus cereus. Isolant numbers 250 Aa and 251 Bb are B. subtilis. The cultural characteristics and physiological tests for the three species of B. cereus are all the same. The two B. subtilis isolants varied in cultural characteristics, litmus milk reactions, hydrolysis of casein, and temperature relationships. Isolant number 251 Bb resembles B. coagulans in milk reactions and casein hydrolysis, although the remaining majority of characteristics are like B. subtilis.

LIST OF ISOLANTS AND SPECIES DESIGNATIONS

Code Number	Species Designation	Page
250 Aa (23°C)	<u>Bacillus subtilis</u>	10
250 Aa (55°C)	Actinomycete	
*250 Ba (23°C)(V.D.)	"Soil Diphtheroid"	14
250 Bb (23°C)	<u>Bacillus cereus</u>	4
251 Aa (23°C)	<u>Bacillus cereus</u>	6
251 Ab (23°C)	<u>Bacillus cereus</u>	8
251 Bb (55°C)	<u>Bacillus subtilis</u>	12

* Failed to grow upon original transfer.

DICHOTOMOUS KEY

I. Actinomycete

250 Aa (55°C)

II. Bacteria

A. Gram positive non-sporulating rods

250 Ba (V.D.) "Soil Diphtheroid" 14

B. Gram positive sporulating rods^{1,2}

I. Sporangia not swollen. Spores ellipsoidal to cylindrical, central to terminal. Spore walls thin and not easily stained. Gram positive.

A. Protoplasm of young cells grown on glucose agar vacuolated if lightly stained. Diameter of vegetative rods is 0.9 micron or more.

1. Acid from mannitol with ammonium salts as source of nitrogen. Acetylmethylcarbinol not produced.

Bacillus megaterium

2. No acid from mannitol with ammonium salts as source of nitrogen. Acetylmethylcarbinol produced.

Bacillus cereus

250 Bb	4
251 Aa	6
251 Ab	8

B. Protoplasm of young cells grown on glucose agar not vacuolated if lightly stained. Diameter of vegetative rods is less than 0.9 micron.

1. Growth on glucose agar as good as or better than on agar. Good growth on soybean agar.

- a. Growth in 7 per cent NaCl agar.
- b. Starch hydrolyzed. Nitrites produced from nitrates.
- c. Good growth under anaerobic conditions in glucose broth; pH of cultures is 5.2 or below. Gas produced from nitrates under alkaline, anaerobic conditions.

Bacillus licheniformis

- cc. Scant if any growth in glucose broth under anaerobic conditions; pH of cultures is higher than 5.2. No gas produced from nitrates under alkaline, anaerobic conditions.

Bacillus subtilis

250 Aa	10
251 Bb	12

¹ Smith, Nathan R., Ruth E. Gordon, and Francis E. Clark. 1952. Aerobic Sporeforming Bacteria. Agriculture Monograph No. 16. U. S. Department of Agriculture.

² Breed, Robert S., E. G. D. Murray, and Nathan R. Smith. 1957. Bergey's Manual of Determinative Bacteriology. 7th Ed. Baltimore, The Williams and Wilkins Company.

Descriptive Chart

<u>250Bb</u> (code number)	<u>Trypticase Soy Agar</u> (medium)	<u>Mexico</u> (source)
<u>Bacillus cereus</u> (name of organism)	<u>28°C.</u> (temperature)	<u>W. B. Bollen</u> (studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

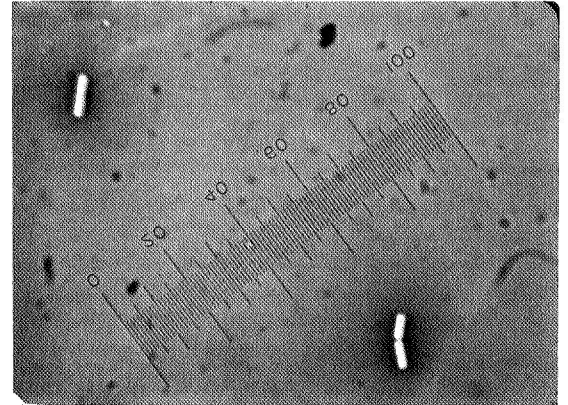
MORPHOLOGY:

Form: rods, ends ROUNDED,
filaments, cocci, spirals,
branching _____
Size: average —
range —
Irregular forms:

GRAM REACTION: **POSITIVE.**

18 hrs:
24 hrs:
48 hrs:

NIGROSIN STAIN:



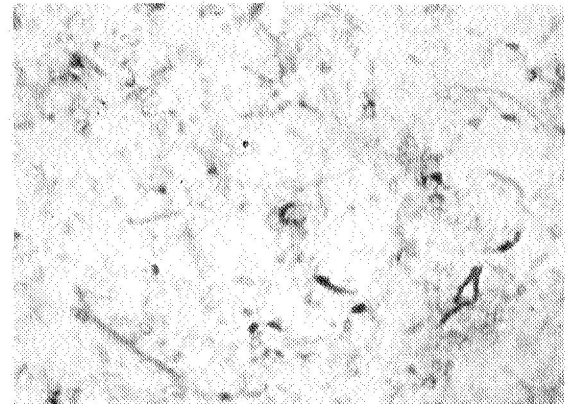
PASTEURIZATION SURVIVAL (85°C, 10 minutes): **POSITIVE.**

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.
Endospores: swollen, not swollen.
Position: central to excentric, terminal, subterminal.
Shape: spherical, ellipsoidal, cylindrical, oval.
size: average —
range —

MOTILITY: age _____.

Flagella:

SPORE STAIN:



OTHER STAINS:

Acid fast:
Capsule:
Glycogen:
Crystalline dextrans:
Fat globules:
Metachromatic granules:

II. CULTURAL CHARACTERISTICS

AGAR STROKE: age 23 DA

Amount of growth: abundant, moderate, scant.

Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.

Consistency: adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.

AGAR COLONIES: age 23 DA

Macroscopic

Size: 4 mm.

Shape: filamentous, irregular, oval, puntiform, round.

Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.

Topography: contoured, rough, smooth, striated, wrinkled.

Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate. FIMBRIATE.

Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.

Optical properties

Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.

Appearance by transmitted light: opaque, translucent, transparent.

Medium: blackened, blued, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium) (color) (CHM No.)

Trypticase soy agar CAMEL 3ie

Potato slant

-
-

NUTRIENT BROTH: age _____

Amount of growth: abundant, moderate, scant.

Surface growth: none, flocculent, membranous, pellicle, ring.

Subsurface growth: none, granular, turbid.

Sediment: none, compact, flaky, flocculent, granular, viscid.

Odor: resembling _____

GELATIN STAB: age _____

Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.

Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: ABUNDANT GROWTH.

Fat agar:

Glucose-nitrate agar: SLIGHT GROWTH.

GROWTH ON GLUCOSE NUTRIENT AGAR BETTER THAN GROWTH ON NUTRIENT AGAR.

ANAEROBIC NITRATE BROTH: SLIGHT GAS 8 DA.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS 250Bb

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, microaerophilic.

CATALASE: positive, negative.

TEMPERATURE RELATIONSHIPS: age 7 DA
Growth at 10°C. -, 20°C. +, 28°C. +, 37°C. +,
45°C. +, 55°C. - → ± 15°C. - 50°C.

SOLE CARBON SOURCE: age _____

Glucose: positive, negative. 1 DA.

Sucrose: positive, negative. _____

Xylose: positive, negative. _____

Citrate: positive, negative. 4 DA.

NH₄⁺ AS SOLE NITROGEN SOURCE: positive,
negative. 5 DA.

MANNITOL: POSITIVE (NO ACID). 5 DA.

REDUCTIONS:

Nitrate: NO₂⁻ +, NH₄⁺ _____, gas _____, negative. 1 DA.

Methylene blue: positive, negative. _____

Selenite: positive, negative. _____

Tellurite: positive, negative. _____

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid _____, alkaline _____, neutral, gas. _____

Sucrose: acid _____, alkaline _____, neutral, gas. _____

Lactose: acid _____, alkaline _____, neutral, gas. _____

Xylose: acid _____, alkaline _____, neutral, gas. _____

Mannitol: acid _____, alkaline, neutral, gas. _____

HYDROLYSIS:

Gelatin: positive, negative. 3 DA.

Casein: positive, negative. 1 DA.

Fat: positive, negative. _____

Starch: positive, negative. 3 DA.

Cellulose: positive, negative. _____

Urea: positive, negative. _____

TOLERANCES:

Salt: 2%—positive, negative. 1 DA.

7%—positive, negative. 10 DA.

10%—positive, negative. 10 DA.

pH: acid _____, alkaline _____.

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral. 6 DA.

Curd: acid, alkaline, absent, gas. 30 DA.

Peptonization: positive, negative. 2 DA.

Reduction: positive, negative. 3 DA.

OTHER REACTIONS:

H₂S from _____: positive,
negative. _____

NH₄⁺ from peptone: positive, negative. _____

Acetylmethylcarbinol: positive, negative. 18 DA.

Indol: positive, negative. 18 DA.

Methyl red: positive, negative. _____

Descriptive Chart

<u>251Aa</u> (code number)	<u>(23°C.TSA) Trypticase Soy Agar</u> (medium)	<u>Mexico</u> (source)
<u>Bacillus cereus</u> (name of organism)	<u>28°C.</u> (temperature)	<u>W. B. Bollen</u> (studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

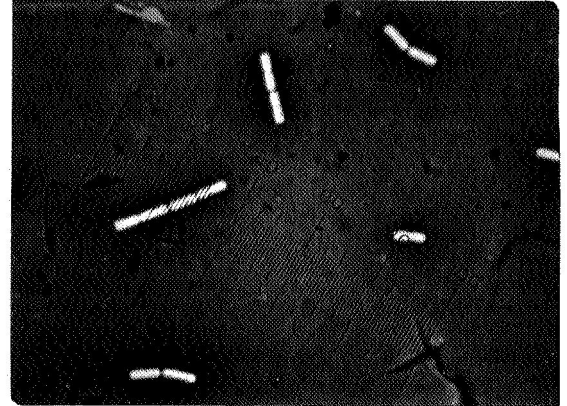
MORPHOLOGY:

Form: rods, ends **ROUNDED**,
filaments, cocci, spirals,
branching _____
Size: *average* -
range -
Irregular forms:

GRAM REACTION: **POSITIVE.**

18 hrs:
24 hrs:
48 hrs:

NIGROSIN STAIN:



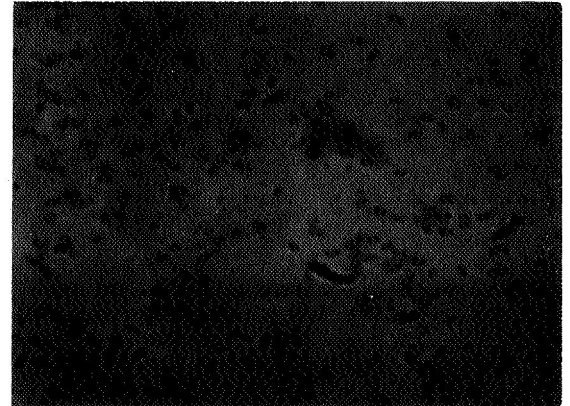
PASTEURIZATION SURVIVAL (85°C, 10 minutes): **POSITIVE.**

Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.*
Endospores: *swollen, not swollen.*
Position: *central to excentric, terminal, subterminal.*
Shape: *spherical, ellipsoidal, cylindrical, oval.*
size: *average* -
range -

MOTILITY: age _____.

Flagella:

SPORE STAIN:



OTHER STAINS:

Acid fast:
Capsule:
Glycogen:
Crystalline dextrans:
Fat globules:
Metachromatic granules:

II. CULTURAL CHARACTERISTICS

AGAR STROKE: age 23 DA.

Amount of growth: abundant, moderate, scant.

Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.

Consistency: adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.

AGAR COLONIES: age 23 DA.

Macroscopic

Size: 4 mm.

Shape: filamentous, irregular, oval, puntiform, round.

Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.

Topography: contoured, rough, smooth, striated, wrinkled.

Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate. FIMBRIATE.

Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.

Optical properties

Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.

Appearance by transmitted light: opaque, translucent, transparent.

Medium: blackened, blued, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium) (color) (CHM No.)

Trypticase soy agar LT IVORY 1 CA

Potato slant

NUTRIENT BROTH: age _____

Amount of growth: abundant, moderate, scant.

Surface growth: none, flocculent, membranous, pellicle, ring.

Subsurface growth: none, granular, turbid.

Sediment: none, compact, flaky, flocculent, granular, viscid.

Odor: resembling _____

GELATIN STAB: age _____

Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.

Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: ABUNDANT.

Fat agar:

Glucose-nitrate agar: NO GROWTH (12 DA.)

GROWTH ON GLUCOSE NUTRIENT AGAR BETTER THAN GROWTH ON NUTRIENT AGAR.

ANAEROBIC NITRATE BROTH: GAS. SLIGHT 8 DA.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS (23⁰C. TSA) ^{251Aa}

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, micro-aerophilic.

CATALASE: positive, negative.

TEMPERATURE RELATIONSHIPS: age 7 DA.

Growth at 10°C. -, 20°C. +, 28°C. +, 37°C. +, 45°C. +, 55°C. -. 15°C. + 30°C. -.

SOLE CARBON SOURCE: age _____

Glucose: positive, negative. 1 DA.

Sucrose: positive, negative. _____

Xylose: positive, negative. _____

Citrate: positive, negative. 4 DA.

NH₄⁺ AS SOLE NITROGEN SOURCE: positive, negative. 5 DA.

MANNITOL : POSITIVE. (NO ACID) 10 DA.

REDUCTIONS:

Nitrate: NO₂⁻ +, NH₄⁺ _____, gas _____, negative. 1 DA.

Methylene blue: positive, negative. _____

Selenite: positive, negative. _____

Tellurite: positive, negative. _____

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid____, alkaline____, neutral____, gas____

Sucrose: acid____, alkaline____, neutral____, gas____

Lactose: acid____, alkaline____, neutral____, gas____

Xylose: acid____, alkaline____, neutral____, gas____

Mannitol: acid____, alkaline____, neutral____, gas____

HYDROLYSIS:

Gelatin: positive, negative. 3 DA.

Casein: positive, negative. 1 DA.

Fat: positive, negative. _____

Starch: positive, negative. 3 DA.

Cellulose: positive, negative. _____

Urea: positive, negative. _____

TOLERANCES:

Salt: 2% positive, negative. 1 DA.

7% positive, negative. 3 DA.

10% positive, negative. 10 DA.

pH: acid____, alkaline_____

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral. 6 DA.

Curd: acid, alkaline, absent, gas. 30 DA.

Peptonization: positive, negative. 2 DA.

Reduction: positive, negative. 3 DA.

OTHER REACTIONS:

H₂S from _____: positive, negative. _____

NH₄⁺ from peptone: positive, negative. _____

Acetylmethylcarbinol: positive, negative. 18 DA.

Indol: positive, negative. 18 DA.

Methyl red: positive, negative. _____

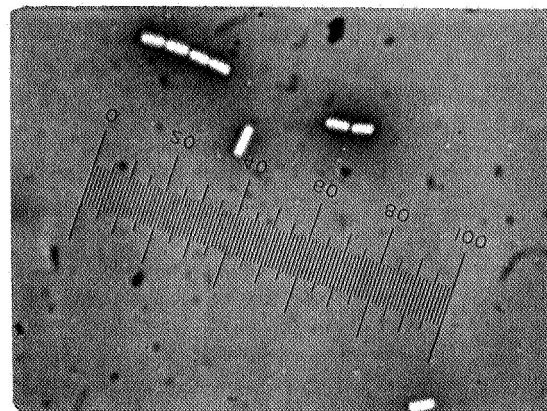
Descriptive Chart

251Ab (code number) (23°C.TSA) Trypticase Soy Agar (medium) Mexico (source)
Bacillus cereus (name of organism) 28°C. (temperature) W. B. Bollen (studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends ROUNDED,
filaments, cocci, spirals,
branching _____
Size: average -
range -
Irregular forms:



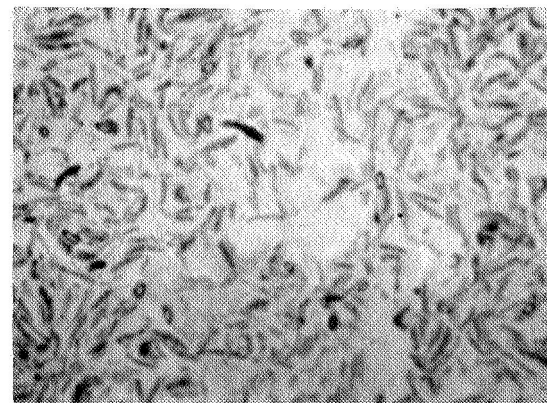
GRAM REACTION: POSITIVE.

18 hrs:
24 hrs:
48 hrs:

NIGROSIN STAIN:

PASTEURIZATION SURVIVAL (85°C, 10 minutes): POSITIVE.

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.
Endospores: swollen, not swollen.
Position: central to excentric, terminal, subterminal.
Shape: spherical, ellipsoidal, cylindrical, oval.
size: average -
range -



MOTILITY: age _____.

Flagella:

SPORE STAIN:

OTHER STAINS:

Acid fast:
Capsule:
Glycogen:
Crystalline dextrans:
Fat globules:
Metachromatic granules:

II. CULTURAL CHARACTERISTICS

AGAR STROKE: age 23 DA.

Amount of growth: abundant, moderate, scant.

Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.

Consistency: adherent, brittle, butyrous, membranous, moist, slimy, soft, tough, viscid, waxy.

AGAR COLONIES: age 23 DA.

Macroscopic

Size: 4 mm.

Shape: filamentous, irregular, oval, puntiform, round.

Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.

Topography: contoured, rough, smooth, striated, wrinkled.

Habit: compact, spreading.

Microscopic (100x)

Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate. FIMBRIATE.

Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.

Optical properties

Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.

Appearance by transmitted light: opaque, translucent, transparent.

Medium: blackened, blued, browned, grayed, greened, yellowed, unchanged.

Chromogenesis:

(medium) (color) (CHM No.)

Trypticase soy agar 2fb BAMBOO

Potato slant

-

-

NUTRIENT BROTH: age _____

Amount of growth: abundant, moderate, scant.

Surface growth: none, flocculent, membranous, pellicle, ring.

Subsurface growth: none, granular, turbid.

Sediment: none, compact, flaky, flocculent, granular, viscid.

Odor: resembling _____

GELATIN STAB: age _____

Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.

Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:

Soybean infusion agar: ABUNDANT GROWTH.

Fat agar:

Glucose-nitrate agar: SLIGHT GROWTH.

GLUCOSE-NUTRIENT AGAR GROWTH BETTER THAN

- NUTRIENT AGAR.

ANAEROBIC NITRATE BROTH: GAS SLIGHT 11 DA.

DNA

G:C _____

G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS (251Ab (23°C. TSA))

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, micro-aerophilic.

CATALASE: positive, negative.

TEMPERATURE RELATIONSHIPS: age 7 DA

Growth at 10°C. -, 20°C. +, 28°C. +, 37°C. +,
45°C. +, 55°C. -. 15°C. + 50°C. -.

SOLE CARBON SOURCE: age _____

Glucose: positive, negative. 1 DA.

Sucrose: positive, negative. _____

Xylose: positive, negative. _____

Citrate: positive, negative. 4 DA.

NH₄⁺ AS SOLE NITROGEN SOURCE: positive, negative. 5 DA.

MANNITOL: POSITIVE (NO ACID). 5 DA.

REDUCTIONS:

Nitrate: NO₂⁻ +, NH₄⁺ _____, gas _____, negative. 1 DA.

Methylene blue: positive, negative. _____

Selenite: positive, negative. _____

Tellurite: positive, negative. _____

OXIDATIVE-FERMENTATIVE REACTIONS

Glucose: acid____, alkaline____, neutral, gas. _____

Sucrose: acid____, alkaline____, neutral, gas. _____

Lactose: acid____, alkaline____, neutral, gas. _____

Xylose: acid____, alkaline____, neutral, gas. _____

Mannitol: acid____, alkaline, neutral, gas. _____

HYDROLYSIS:

Gelatin: positive, negative. 3 DA.

Casein: positive, negative. 1 DA.

Fat: positive, negative. _____

Starch: positive, negative. 3 DA.

Cellulose: positive, negative. _____

Urea: positive, negative. _____

TOLERANCES:

Salt: 2%—positive, negative. 1 DA.

7%—positive, negative. 10 DA.

10%—positive, negative. 10 DA.

pH: acid____, alkaline_____

LITMUS MILK REACTIONS:

Reaction: acid, alkaline, neutral. 27 DA.

Curd: acid, alkaline, absent, gas. 30 DA.

Peptonization: positive, negative. 2 DA.

Reduction: positive, negative. 3 DA.

OTHER REACTIONS:

H₂S from _____: positive, negative. _____

NH₄⁺ from peptone: positive, negative. _____

Acetylmethylcarbinol: positive, negative. 18 DA.

Indol: positive, negative. 18 DA.

Methyl red: positive, negative. _____

Descriptive Chart

250Aa (23°C. TSA) Trypticase Soy Agar Mexico
(code number) (medium) (source)

Bacillus subtilis 28°C. W. B. Bollen
(name of organism) (temperature) (studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

MORPHOLOGY:

Form: rods, ends ROUNDED,
filaments, cocci, spirals,
branching _____.

Size: average —
range —

Irregular forms:

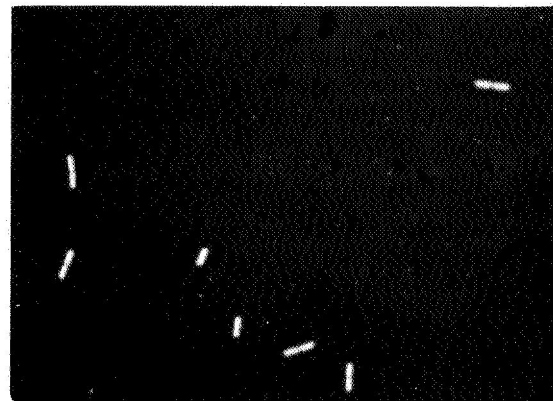
GRAM REACTION: POSITIVE.

18 hrs:

24 hrs:

48 hrs:

NIGROSIN:



PASTEURIZATION SURVIVAL (85°C, 10 minutes): POSITIVE.

Sporangia: none, rods, spindles, elliptical, clavate, drumstick.

Endospores: swollen, not swollen.

Position: central to excentric, terminal, subterminal.

Shape: spherical, ellipsoidal, cylindrical, oval.

size: average —
range —

MOTILITY: age _____.

Flagella:

OTHER STAINS:

Acid fast:

Capsule:

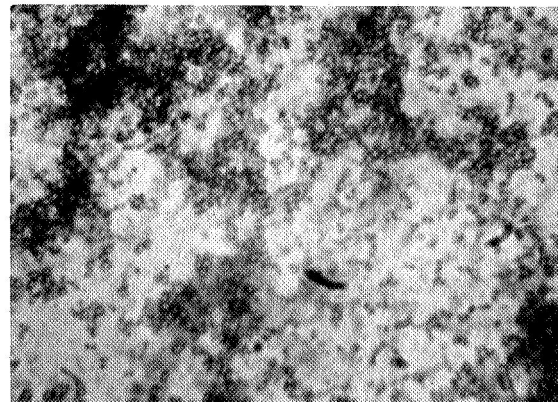
Glycogen:

Crystalline dextrans:

Fat globules:

Metachromatic granules:

SPORE:



II. CULTURAL CHARACTERISTICS

AGAR STROKE: age 23 DA.
 Amount of growth: abundant, moderate, scant.
 Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.
 Consistency: adherent, brittle, buturous, membranous, moist, slimy, soft, tough, viscid, waxy.

AGAR COLONIES: age 23 DA.
 Macroscopic
 Size: 3 mm.
 Shape: filamentous, irregular, oval, puntiform, round.
 Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.
 Topography: contoured, rough, smooth, striated, wrinkled.
 Habit: compact, spreading.
 Microscopic (100x)
 Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.
 Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.
 Optical properties
 Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.
 Appearance by transmitted light: opaque, translucent, transparent.
 Medium: blackened, blue, browned, grayed, greened, yellowed, unchanged.
 Chromogenesis:
 (medium) (color) (CHM No.)
 Trypticase soy agar 2gc BAMBDO
 Potato slant
 -
 -

NUTRIENT BROTH: age _____
 Amount of growth: abundant, moderate, scant.
 Surface growth: none, flocculent, membranous, pellicle, ring.
 Subsurface growth: none, granular, turbid.
 Sediment: none, compact, flaky, flocculent, granular, viscid.
 Odor: resembling _____

GELATIN STAB: age _____
 Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.
 Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:
 Soybean infusion agar: ABUNDANT GROWTH.
 Fat agar:
 Glucose-nitrate agar: GROWTH.
GLUCOSE-NUTRIENT AGAR GROWTH BETTER THAN NUTRIENT AGAR.
ANAEROBIC NITRATE BROTH: NO GAS: 23 DA.

DNA
 G:C _____
 G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, micro-aerophilic.

CATALASE: positive, negative.

TEMPERATURE RELATIONSHIPS: age 7 DA.
 Growth at 10°C. -, 20°C. +, 28°C. +, 37°C. +, 45°C. +, 55°C. -. 15°C. +. 50°C ±.

SOLE CARBON SOURCE: age _____
 Glucose: positive, negative. 1 DA.
 Sucrose: positive, negative. _____
 Xylose: positive, negative. _____
 Citrate: positive, negative. 4 DA.
 NH₄⁺ AS SOLE NITROGEN SOURCE: positive, negative. 5 DA.
MANNITOL: POSITIVE. (NO ACID) 1 DA.

REDUCTIONS:
 Nitrate: NO₂⁻ +, NH₄⁺ _____, gas _____, negative. 1 DA.
 Methylene blue: positive, negative. _____
 Selenite: positive, negative. _____
 Tellurite: positive, negative. _____
 -

OXIDATIVE-FERMENTATIVE REACTIONS
 Glucose: acid _____, alkaline _____, neutral, gas. _____
 Sucrose: acid _____, alkaline _____, neutral, gas. _____
 Lactose: acid _____, alkaline _____, neutral, gas. _____
 Xylose: acid _____, alkaline _____, neutral, gas. _____
 Mannitol: acid _____, alkaline _____, neutral, gas. _____
 -

HYDROLYSIS:
 Gelatin: positive, negative. 3 DA.
 Casein: positive, negative. 3 DA.
 Fat: positive, negative. _____
 Starch: positive, negative. 3 DA.
 Cellulose: positive, negative. _____
 Urea: positive, negative. _____

TOLERANCES:
 Salt: 2% positive, negative. 1 DA.
 7% positive, negative. 3 DA.
 10% positive, negative. 3 DA.
 pH: acid _____, alkaline _____

LITMUS MILK REACTIONS:
 Reaction: acid, alkaline, neutral. 21 DA.
 Curd: acid, alkaline, absent, gas. 30 DA.
 Peptonization: positive, negative. 6 DA.
 Reduction: positive, negative. 3 DA.

OTHER REACTIONS:
 H₂S from _____: positive, negative. _____
 NH₄⁺ from peptone: positive, negative. _____
 Acetylmethylcarbinol: positive, negative. 18 DA.
 Indol: positive, negative. 18 DA.
 Methyl red: positive, negative. _____

OREGON STATE UNIVERSITY
DEPARTMENT OF MICROBIOLOGY
(JPL-NASA)

Descriptive Chart

251Bb (code number) (55°C.TSA) (medium) Trypticase Soy Agar Mexico (source)

Bacillus subtilis (name of organism) 28°C. (temperature) W. B. Bollen (studied by)

I. STAINING & MORPHOLOGICAL CHARACTERISTICS

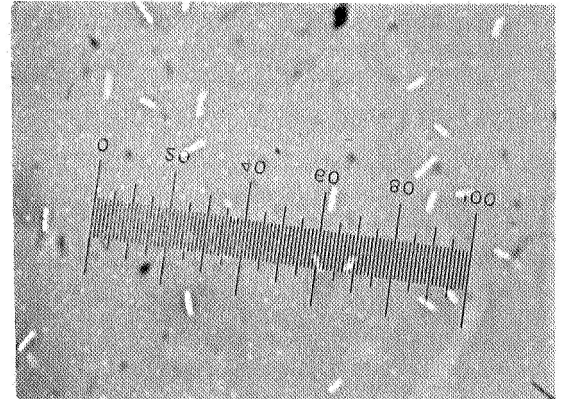
MORPHOLOGY:

Form: rods, ends ROUNDED,
filaments, cocci, spirals,
branching _____
Size: *average* -
range -
Irregular forms:

GRAM REACTION: **POSITIVE.**

18 hrs:
24 hrs:
48 hrs:

NIGROSIN STAIN:



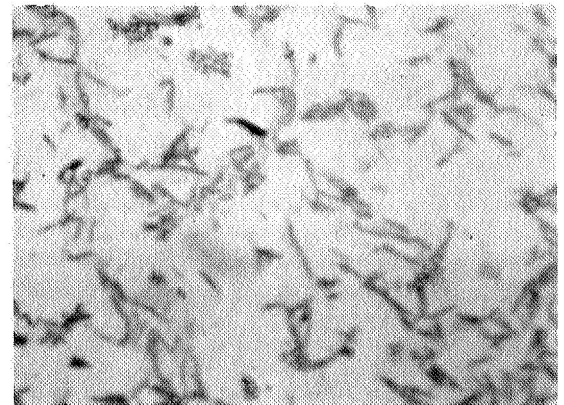
PASTEURIZATION SURVIVAL (85°C, 10 minutes): **POSITIVE.**

Sporangia: *none*, rods, *spindles, elliptical, clavate, drumstick.*
Endospores: *swollen*, not swollen.
Position: central to excentric, *terminal, subterminal.*
Shape: *spherical, ellipsoidal, cylindrical, oval.*
size: *average* -
range -

MOTILITY: age _____.

Flagella:

SPORE STAIN:



OTHER STAINS:

Acid fast:
Capsule:
Glycogen:
Crystalline dextrans:
Fat globules:
Metachromatic granules:

II. CULTURAL CHARACTERISTICS

AGAR STROKE: age 22 DA
 Amount of growth: abundant, moderate, scant.
 Form: aborescent, beaded, echinulate, effuse, filiform, rhizoid, spreading.
 Consistency: adherent, brittle, buturous, membranous, moist, slimy, soft, tough, viscid, waxy.

AGAR COLONIES: age 22 DA
 Macroscopic
 Size: 4 mm.
 Shape: filamentous, irregular, oval, puntiform, round.
 Elevation: beveled, convex, effuse, flat, papillate, pulvinate, raised, rugose, umbonate.
 Topography: contoured, rough, smooth, striated, wrinkled.
 Habit: compact, spreading.
 Microscopic (100x)
 Margin: Ciliate, cleft, crenate, entire, erose, granular, lobed, rhizoid, undulate.
 Internal structure: amorphous, dense, filamentous, granular (fine, coarse), interlaced, striated.
 Optical properties
 Appearance by reflected light: dull, fluorescent, glistening, iridescent, opalescent.
 Appearance by transmitted light: opaque, translucent, transparent.
 Medium: blackened, blueed, browned, grayed, greened, yellowed, unchanged.
 Chromogenesis:
 (medium) (color) (CHM No.)
 Trypticase soy agar 2 CA LT. IVORY
 Potato slant
 -
 -

NUTRIENT BROTH: age _____
 Amount of growth: abundant, moderate, scant.
 Surface growth: none, flocculent, membranous, pellicle, ring.
 Subsurface growth: none, granular, turbid.
 Sediment: none, compact, flaky, flocculent, granular, viscid.
 Odor: resembling _____

GELATIN STAB: age _____
 Liquefaction: none, crateriform, infundibuliform, napiform, saccate, stratiform.
 Rate: fast, moderate, slow.

OTHER GROWTH CHARACTERISTICS:
 Soybean infusion agar: ABUNDANT GROWTH.
 Fat agar:
 Glucose-nitrate agar: GROWTH.
GROWTH ON GLUCOSE-NUTRIENT AGAR & NUTRIENT
AGAR ABUNDANT.

ANAEROBIC NITRATE BROTH: SLIGHT GAS 11 DA.

DNA
 G:C _____
 G+C _____ moles %

III. PHYSIOLOGICAL CHARACTERISTICS

RELATIONSHIP TO O₂: aerobic, anaerobic, facultative, microaerophilic.
 CATALASE: positive, negative.

TEMPERATURE RELATIONSHIPS: age 7 DA
 Growth at 10°C. -, 20°C. +, 28°C. +, 37°C. +,
 45°C. +, 55°C. + 15°C. = : 60°C. =.

SOLE CARBON SOURCE: age _____
 Glucose: positive, negative. 1 DA.
 Sucrose: positive, negative. _____
 Xylose: positive, negative. _____
 Citrate: positive, negative. 4 DA.
 NH₄⁺ AS SOLE NITROGEN SOURCE: positive, negative. 5 DA.

MIANNITOL: POSITIVE (NO ACID) 5 DA.
 REDUCTIONS:
 Nitrate: NO₂⁻ +, NH₄⁺ _____, gas _____, negative. 1 DA.
 Methylene blue: positive, negative. _____
 Selenite: positive, negative. _____
 Tellurite: positive, negative. _____

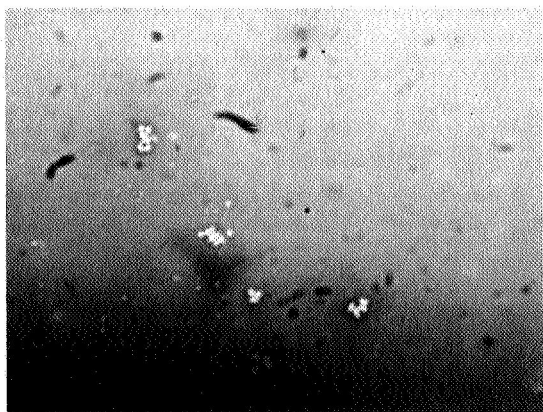
OXIDATIVE-FERMENTATIVE REACTIONS
 Glucose: acid____, alkaline____, neutral, gas. _____
 Sucrose: acid____, alkaline____, neutral, gas. _____
 Lactose: acid____, alkaline____, neutral, gas. _____
 Xylose: acid____, alkaline____, neutral, gas. _____
 Mannitol: acid____, alkaline, neutral, gas. _____

HYDROLYSIS:
 Gelatin: positive, negative. 3 DA.
 Casein: positive, negative. SLIGHT 9 DA.
 Fat: positive, negative. _____
 Starch: positive, negative. 6 DA.
 Cellulose: positive, negative. _____
 Urea: positive, negative. _____

TOLERANCES:
 Salt: 2% positive, negative. 1 DA.
 7% positive, negative. 3 DA.
 10% positive, negative. 5 DA.
 pH: acid____, alkaline_____

LITMUS MILK REACTIONS:
 Reaction: acid, alkaline, neutral. 21 DA.
 Curd: acid, alkaline, absent, gas. WHEY SEP. 6 DA.
 Peptonization: positive, negative. 30 DA.
 Reduction: positive, negative. 3 DA.

OTHER REACTIONS:
 H₂S from _____: positive, negative. _____
 NH₄⁺ from peptone: positive, negative. _____
 Acetylmethylcarbinol: positive, negative. 18 DA.
 Indol: positive, negative. 18 DA.
 Methyl red: positive, negative. _____

PHOTOMICROGRAPH OF NON-VIABLE ORIGINAL CULTURE 250B_a (V.D.)

NIGROSIN STAIN

1000X